# Survival of Colletotrichum graminicola in Corn Kernels

H. L. Warren

Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Cooperative investigation of Agricultural Research Service, U.S. Department of Agriculture and Purdue University, West Lafayette, IN 47907.

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## ABSTRACT

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*Colletotrichum graminicola,* which causes anthracnose, survived on corn kernels at 4 C more than 3 yr. Viability of *C. graminicola* was stable on naturally infected kernels, but declined on artificially infected kernels during the 3-yr study. *Fusarium moniliforme, Aspergillus flavus,* and *Penicillium* spp. were isolated more frequently from *C. graminicola*-infected kernels after 24 mo in storage than after earlier sampling dates. The prevalence of *C. graminicola* varied from year to year. Infection of inbred kernels ranged from 0 to 9%, whereas infection of a sweet corn hybrid and an advanced breeding line reached 39 and 51%, respectively.

*Additional key words:* maize, *Zea mays,* kernel infection.

In 1957, Messiaen and Lafon (1) reported infection of dent corn (Zea *mays* L.) kernels by artificial inoculation with *Colletotrichum graminicola* (Ces.) G. W. Wils. In 1973, Warren et al. reported the natural occurrence of this disease on sweet corn (5) and in 1975, the disease was reported on dent corn kernels (3). A low percentage of kernel infection was reported in areas where anthracnose was common on the foliage or stalks. The incidence of infected kernels rarely exceeded 2% and infection in seed lots was found only in areas reporting incidence of foliar or stalk infection. However, infected kernels may have been overlooked because *C. graminicola* grows slowly on potato-dextrose agar. *Colletotrichum graminicola* can be carried on infected corn kernels. Reduced stands (seedling blight) appears to be the most serious problem associated with the infected kernels (3). Acervuli form on the pericarp; however, little is known about the pathogen-kernel relationship. There has been little or no investigation on survival of *C. graminicola* on corn kernels in storage. This report describes the effect of cold storage on survival of the pathogen from several sources and the prevalence of the fungus on kernels during several seasons.

## MATERIALS AND METHODS

Kernels from seed lots from a field with foliar and stalk infection were examined for damage caused by *C. graminicola.* Seed lots were hand-harvested in September and October, dried to 15% moisture at 37 C for 5-7 days, stored at 4 C, and examined annually in November. Moisture content was determined on a fresh weight basis. After storage, 100 to 200 kernels of each lot were surface-disinfected in 1% sodium hypochlorite for 2 min, rinsed with sterile water, blotted with sterile filter paper, and lots of 10 kernels were placed in petri plates that contained potato-dextrose agar (PDA). The plates were incubated at 24 C under light (4,500 lux) for 10 days, and the percentage of infected kernels was determined. The test was repeated one or more times.

In a second trial, corn ears were inoculated by injecting 1 ml of a spore suspension of *C. graminicola* (20,000 spores/ml) into the ear sheath 30 days after pollination. Care was taken to prevent physical damage of the kernels. After harvest, the kernels were treated as described and plated on PDA to obtain the percentage of infected kernels. The remaining kernels were stored at 4 C for further testing.

In another test, corn kernels with visual signs of the fungus were selected, examined under low magnification, and those with acervuli were plated on PDA after 12 and 24 mo in storage at 4 C. From four inbred and hybrid lines, corn kernels were collected from 1972-1975 to ascertain the prevalence of *C. graminicola* each year. These lines were hand-harvested in October and examined 4-8 wk after storage at 4 C. In 1972 and 1974, kernels were plated on PDA immediately after harvest and others 6 wk later. No differences were observed in percentages of infected kernels when examined immediately or after storage for 6 wk; thus, only data from kernels in storage are presented.

## RESULTS

**Prevalence of Colletotrichum graminicola on corn kernels.**—Corn kernels assayed 4-8 wk after harvest had a higher visual rating (presence of acervuli) than those assayed 5-7 days after harvest. The acervuli were darker and streaks were more pronounced on kernels dried to

15% moisture content and examined 4-8 wk after storage than those not dried and examined 5-7 days after harvest. However, when assayed on PDA, the percentage of infected kernels was similar for both storage periods. The percentage of infected kernels for most lines was higher in 1973 and 1974 than in the preceding year (Table 1). The increase in kernel infection of E43-25 and Jubliee paralleled an observed increase in foliar and stalk infection from 1972 to 1975. The incidence of kernel infection on the dent corn breeding line E43-25, and the susceptible sweet corn hybrid Jubilee, was 39 and 19% in 1974 as compared to 6 and 8% in 1972, respectively. However, on dent corn inbreds, infected kernels never exceeded 9%. These inbreds, hybrids, and breeding lines were grown in areas near the plot inoculated with *C. graminicola*. This may account for the high incidence of *C. graminicola* on these entries. Other inbred lines in these tests that showed 4% or more infected kernels in 1 yr or more were B 37 and Oh 43.

TABLE 1. Prevalence of *Colletotrichum graminicola* on corn kernels on selected inbreds from 1972 - 1975

| Corn pedigree | Infection (%) by year | | | |
|---|---|---|---|---|
| | 1972 | 1973 | 1974 | 1975 |
| Mo 940 | 3 | 8 | 9 | 3 |
| E 43-25[a] | 6 | 22 | 39 | 17 |
| H 59 | 4 | 4 | 8 | 5 |
| Jubilee[a] | 8 | 16 | 19 | 12 |
| Oh 43 | 0 | 4 | 1 | 6 |
| B 37 | 1 | 5 | 1 | 4 |
| A 632 | 0 | 0 | 0 | 0 |
| H 84 | 0 | 0 | 0 | 0 |

[a]Corn E 43-25 is an advanced breeding line and Jubilee is a sweet corn hybrid.

TABLE 2. Seed germination and frequency of isolation of *Colletotrichum graminicola* from corn kernels grown in areas with *Colletotrichum graminicola* infestation from 1972 - 1975

| Corn pedigree | Percentage of seeds infected and seed germination per years in storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | Infection (%) | | | | Germination (%) | | |
| | 1 yr | 2 yr | 3 yr | 4 yr | 1 yr | 2 yr | 3 yr |
| Mo 940 | 8 | 6 | 6 | 5 | 92 | 87 | 82 |
| H 59 | 6 | 6 | 4 | 5 | 95 | 90 | 90 |
| B 37 | 5 | 5 | 4 | 4 | 98 | 92 | 86 |
| Oh 43 | 4 | 3 | 4 | 3 | 96 | 90 | 86 |
| E 43-25 | 51 | 50 | 41 | 36 | 86 | 76 | 75 |
| Jubilee | 39 | 37 | 28 | 18 | 80 | 70 | 40 |

TABLE 3. Effect of time on viability of *Colletotrichum graminicola* and seed germination by kernels artificially inoculated and stored at 4 C

| Corn inbred | Percentage of seeds infected and seed germination per years in storage | | | | | |
|---|---|---|---|---|---|---|
| | Infection (%) | | | Germination (%) | | |
| | 0 yr | 1 yr | 2 yr | 0 yr | 1 yr | 2 yr |
| Mo 940 | 90 | 85 | 76 | 76 | 50 | 24 |
| H 59 | 92 | 78 | 66 | 79 | 48 | 28 |
| Control Mo 940 | 3 | 2 | 2 | 98 | 96 | 95 |
| Control H 59 | 2 | 1 | 2 | 96 | 94 | 94 |

**Viability on naturally infected kernels.**—Infection of dent corn kernels under field conditions rarely exceeded 4%. The percentage of infected kernels from these fields remained fairly constant over a 3-yr period in storage at 4 C (Table 2). In particular, those sweet corn and breeding lines that initially had a high percentage of infected kernels commonly showed lower percentages of infection after 36 mo in storage. However, *Fusarium moniliforme, Penicillium* spp., *Aspergillus flavus,* and other storage fungi were isolated more frequently 24 mo or more in storage than at shorter storage times. The presence of these organisms may account for the inability to isolate *C. graminicola* from previously-infected kernels.

Germination percentages of both sweet and dent corn decreased with time in storage up to 3 yr, and was associated with an increase in the number of kernels with discolored germs. After 2 yr, approximately 25% of the kernels were viable. The decrease in germination was greater in infected kernels than in apparently-healthy kernels. This indicates deterioration in storage.

**Viability of Colletotrichum graminicola on corn kernels inoculated artificially.**—Ears were inoculated with a spore suspension to test whether *C. graminicola* causes severe kernel infection in the field. The inoculum was placed near the tip of the ear and infected kernels were observed at the butt of the ear. Symptoms observed on artificially-infected kernels were similar to those on naturally infected kernels and acervuli were visible or in the incipient stage of development. More than 90% infection was detectable on the kernels at storage time, but after 2 yr in storage at 4 C the percentage of kernels with viable *C. graminicola* was only about 75% or less (Table 3). Germination of infected kernels was lower than apparently healthy kernels at storage time, and continued to decrease with time in storage (Table 3). Fungi other than *C. graminicola* were isolated more frequently after 1 and 2 yr of storage than immediately after harvest.

## DISCUSSION

The increase in kernel infection in 1974 and 1975 correlates well with increases in foliar and stalk infection observed in those years. Since 1972, anthracnose has been found in corn over wider geographical areas in the corn belt states and southeastern USA with an apparent increase in virulence of the pathogen (6). Before 1963 (7), pathogenicity was established only on wounded leaves. The difference in virulence could be due to inoculation techniques or genotypes tested. Since 1963, the pathogen has been reported to cause infection on all plant parts (4).

In previous studies, kernel infection of commercial seed lots was low. Seed lots from areas with *C. graminicola* on the foliage had 2% or less infected kernels (3). The present study indicates that infected kernels on some inbreds and breeding lines may reach nearly 50%. These inbreds were grown near the test plot for *C. graminicola* where the inoculum potential was high. This may account for the

higher percentage of infected kernels in this test than in previous studies.

During storage of naturally-infected kernels, parasitism indirectly benefits *C. graminicola*. The fungus can survive for more than 1 yr in kernels and seedling derived from infected kernels usually are infected (3). These infected seedlings may serve as a source of inoculum. Viability of the fungus decreases with time in storage, and conidia from acervuli lose viability within 6 wk (2). Fungal growth from the kernel is presumed to be from stroma or hyphae embedded in the endosperm. Prolonged storage leads to a decrease of *C. graminicola* and an increase in other storage fungi. *Fusarium moniliforme* may restrict growth of *C. graminicola* or, since *C. graminicola* grows slowly, other fungi may become dominant after prolonged storage.

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